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A new species of *Pyricularia* on *Commelina communis*

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Abstract – *Pyricularia commelinicola* sp. nov., causing circular leaf spots, was found on *Commelina communis* in Korea. The fungus is described, illustrated, and compared with the previously known *Pyricularia* species on the genus *Commelina*. The phylogenetic relationship of the fungus with other *Pyricularia* species is discussed.

Key words – blast, *Pyricularia oryzae* var. *commelinae*, *Magnaporthe*, anamorphic fungi, phylogeny

Introduction

Members of the genus *Pyricularia* (anamorphic *Magnaporthe*) cause blast disease, mostly on monocotyledonous plants. Of the approximately 70 taxa in the genus, *P. grisea* Sacc. and *P. oryzae* Cavara are the best known species. They occur on family *Poaceae* and were originally described on *Digitaria sanguinalis* and *Oryza sativa*, respectively. Many other species are associated with host plants belonging to especially *Cannaceae*, *Commelinaceae*, *Cyperaceae*, *Musaceae*, and *Zingiberaceae*. The taxonomy of *Pyricularia* species has been based mainly on host range and morphology of conidia, conidiophores, and appressoria. Recently, molecular analysis of DNA sequence data has become important in the study of the genus. The morphological similarity of *P. grisea* and *P. oryzae* has caused taxonomic controversy as to whether the two taxa are synonymous (e.g. Rossman et al. 1990). The incompatible results of mating experiments (Hebert 1971, Yaegashi & Udagawa 1978, Kato et al. 2000) have made it more difficult to resolve the taxonomic problem. Nevertheless, molecular analyses (Borromeo et al. 1993, Kato et al. 2000) have provided evidence for genetic separation of the two taxa. A significant contribution to the taxonomic resolution came from the multilocus genealogical approach of Couch & Kohn (2002), although to some

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Table 1. Sequence data used in the molecular analysis

Species	Host Species	Isolate No	GenBank No
Pyricularia angulata	*Musa sapientum*	NBRC9625	AY265322
Pyricularia costina	*Alpinia malaccensis*	ICMP14609	AY265329
Pyricularia grisea	*Digitaria sanguinalis*	MAFF240217	AB274428
Pyricularia grisea	*Digitaria sanguinalis*	MAFF240218	AB274429
Pyricularia grisea	*Digitaria horizontalis*	MAFF240210	AB274430
Pyricularia higginsii	*Microlaena avenacea*	ICMP14620	AY265325
Pyricularia higginsii	*Microlaena avenacea*	ICMP14707	AY265326
Pyricularia juncicola	*Carex* sp.	ICMP14625	AY265320
Pyricularia juncicola	*Uncinia* sp.	P17	AY265321
Pyricularia oryzae	*Oryza sativa*	MAFF235005	AB274418
Pyricularia oryzae	*Oryza sativa*	MAFF235006	AB274419
Pyricularia oryzae	*Oryza sativa*	MAFF235003	AB274420
Pyricularia oryzae	*Oryza sativa*	PO-02-7306	AB274421
Pyricularia oryzae	*Setaria italica*	MAFF240214	AB274422
Pyricularia oryzae	*Eleusine coracana*	MAFF240215	AB274423
Pyricularia oryzae	*Eleusine coracana*	MAFF240216	AB274425
Pyricularia oryzae	*Avena sativa*	MAFF240213	AB274424
Pyricularia zingiberis	*Zingiber mioga*	MAFF240222	AB274433
Pyricularia zingiberis	*Zingiber mioga*	MAFF240223	AB274434
Pyricularia zizaniicola	*Zizania latifolia*	MAFF240219	AB274431
Pyricularia zizaniicola	*Zizania latifolia*	MAFF240220	AB274432
Pyricularia commelinicola	*Commelina communis*	KACC43081	FJ850122*
Pyricularia commelinicola	*Commelina communis*	KACC43869	FJ850123*
Pyricularia commelinicola	*Commelina communis*	KACC43966	FJ850124*
Pyricularia commelinicola	*Commelina communis*	KACC44083	FJ850125*

* Sequences obtained in the present study

extent debate and confusion over the taxonomic status of the two species still remain. Recently, Hirata et al. (2007) emphasized that combining morphological or biological species criteria with a phylogenetic species concept is required to determine current species delimitation in *Pyricularia*.

During extensive surveys of phytopathogenic fungi in Korea, symptoms of circular zonate leaf spots were observed on *Commelina communis*. The presence of the typical pyriform conidia placed the fungal pathogen unambiguously in *Pyricularia*. We compared the fungus morphologically with three *Pyricularia* taxa recorded on other *Commelina* species and molecularly analyzed the ITS rDNA region to clarify phylogenetic relationships among the present fungus and other *Pyricularia* species for which ITS sequence data were available from GenBank. On the basis of morphological and molecular data, the fungus isolated from *C. communis* is considered to represent a new species of *Pyricularia*, which is described and illustrated below.

Materials and methods

Nine samples of a *Pyricularia* fungus were collected on *Commelina communis* from July to October in Korea. Fresh collections and herbarium specimens were used for morphological observation. For fresh specimens a small piece of living tissue containing fungal structures was mounted in a drop of water for microscopic examination, while dried specimens were rehydrated in 3% KOH solution and then examined. For each sample, 40–50 conidiophores and conidia were measured at a magnification of 200× and 400× using an eye-piece micrometer and an Olympus BX51 microscope (Olympus, Tokyo, Japan). Images were obtained using a Zeiss Axio imager microscope (Carl Zeiss, Göttingen, Germany).

Cultures were derived from single conidia and grown on potato dextrose agar (PDA). After four weeks, mycelia harvested from the surface of the colonies, were used for genomic DNA extraction following method of Lee & Taylor (1990). The primers ITS1 and ITS4 were used to amplify the ITS rDNA region (White et al. 1990). The obtained PCR products were purified using a QIAquick gel extraction kit (Qiagene, Valencia, CA, USA), and then directly sequenced on an ABI Prism TM 377 automatic DNA sequencer (Applied Biosystems, Foster City, CA, USA), using BigDye™ cycle sequencing kit version 3.1 (Applied Biosystems), with the same primers used for PCR. The ITS rDNA sequences were edited using the DNASTAR computer package version 5.05 (Lasergene, Madison, WI). The newly obtained ITS sequences of four isolates were deposited in GenBank, from which 21 sequences of *Pyricularia* spp. were retrieved for comparison (Table 1). A phylogenetic tree was constructed by neighbor-joining method using MEGA4 version 4.0 (Tamura et al. 2007). The relative robustness of the individual branches was estimated by bootstrapping

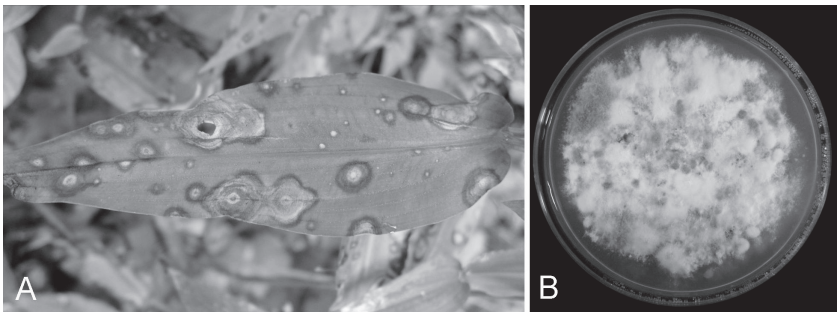

Fig. 1. A - Symptoms of *Pyricularia commelinicola* on *Commelina communis*. B - Colony of *P. commelinicola* after four weeks of incubation on PDA.

using 1000 replicates. A sequence of *Gaeumannomyces amomi* (AY265317), a member of the *Magnaporthaceae*, was used as an outgroup.

Taxonomy

Pyricularia commelinicola M.J. Park & H.D. Shin, **sp. nov.** Figs. 1–2

MycoBank MB513167

Maculae amphigenae, discretae, orbiculares vel suborbiculares, 6–10 mm diam., concentricae; ad centrum pallide brunneae vel griseae, margines brunneae vel atrobrunneae cum halores flavidae. Conidiophora hypophylla, floccosa, raro ramosa, ad 7-septata, recta vel geniculata, hyalina, laevia, 150–530 µm longa, 4–6 µm lata, ad basim 10–12 µm lata. Cellulae conidiogenae terminales vel intercalares, cylindricae, geniculatae, denticulatae; cicatrices conidiales conspicuae, leniter incrassatae, fuscatae. Conidia solitaria, piriformis vel obclavata, hyalina, laevia, 2-septata, 27–42(–45) × 10–12.5 µm, hila eminentia. Coloniae in agaro decocto tuberorum, 60 mm diam. in 14 dies ad 25°C, effusae, albae vel pallide griseae.

Holotype – On living leaves of *Commelina communis* L. (*Commelinaceae*), KOREA, Hongcheon, Bukbang-ri, 37°48'1" N, 127°51'9" E, 9 September 2007, H.D. Shin & M.J. Park, KUS-F 22838 (culture ex-type: KACC43081)

Etymology – The epithet refers to the host plant, *Commelina*.

Leaf spots amphigenous, scattered, sometimes confluent, circular to subcircular, 6–10 mm diam., zonate, centre pale brownish to grayish with brownish to dark brownish margin, often surrounded by yellow halo. Conidiophores hypophyllous, floccose, rarely branched, up to 7-septate, straight or geniculate, hyaline, smooth, 150–530 µm long, 4–6 µm thick, 10–12 µm wide at the bulbous base. Conidiogenous cells terminal or intercalary, cylindrical, geniculate, denticulate; conidiogenous scars conspicuous, slightly thickened, darkened. Conidia solitary, pyriform to obclavate, hyaline, smooth, 2-septate, 27–42(–45) × 10–12.5 µm (mean = 35.3 × 11.2 µm), length/width (l/w) ratio 2.4–4.3 (mean = 3.1), hilum protuberant. Colonies on PDA reaching 60 mm in 14 days at 25°C, effuse, whitish to pale grayish.

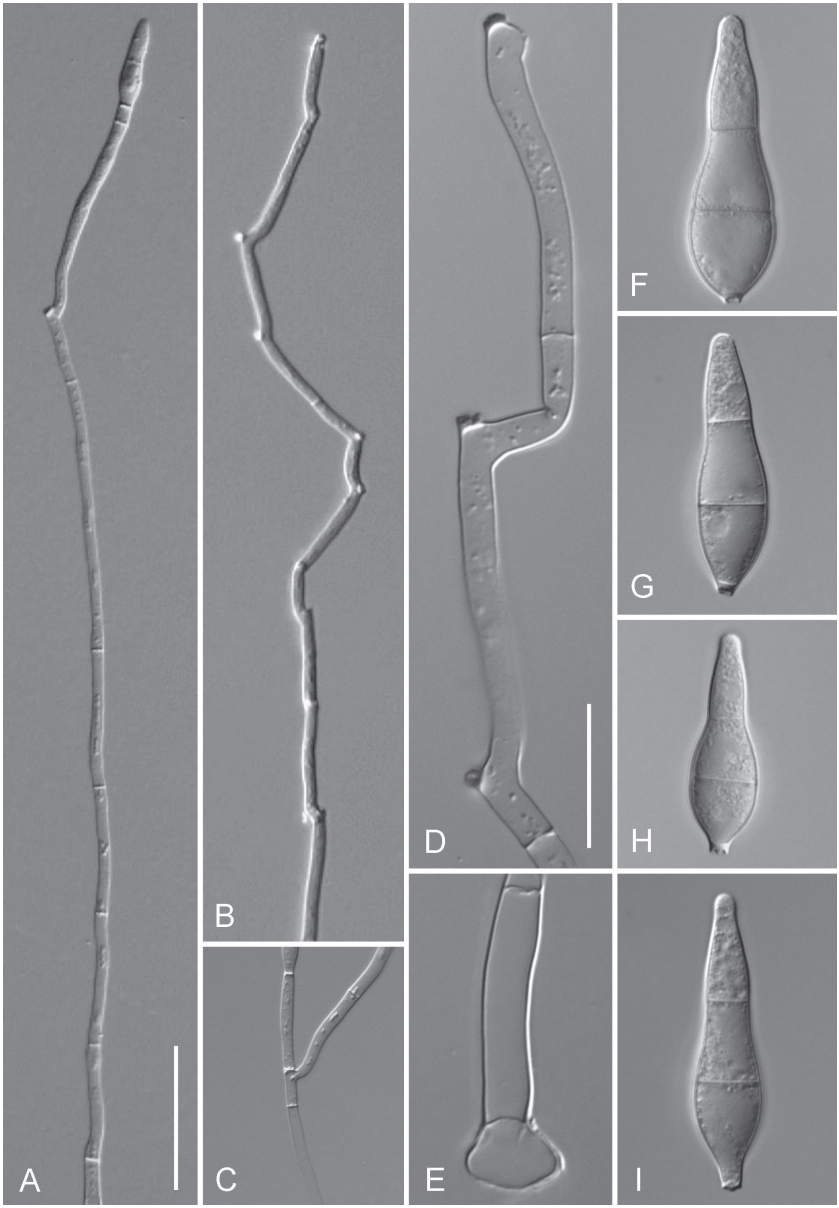

Fig. 2. *Pyricularia commelinicola*: A - Conidiophore producing an immature conidium. B - Geniculate conidiophore. C - Branched part of conidiophore. D - Apical part of conidiophore with conidiogenous scars. E - Basal part of conidiophore. F–I - Conidia.

Scale bars: A–C = 50um, D–I = 20um.

Additional Specimens Examined – Korea, Anyang, Kwanak arboretum, 37°25'8" N, 126°56'5" E, 8 October 1999, H.D. Shin, KUS-F16992; Korea, Chuncheon, Bongmyeong-ri, 37°46'49" N, 127°48'55" E, 1 October 2003, H.D. Shin, KUS-F19767; Korea, Hoengseong, Seowon-myon, 37°31'33" N, 127°52'29" E, 3 August 2007, H.D. Shin & M.J. Park, KUS-F22751; Korea, Chuncheon, Bongmyeong-ri, 37°46'49" N, 127°48'55" E, 20 August 2007, H.D. Shin, KUS-F22768; Korea, Yangpyeong, Experimental Forest of Korea University, 37°30'12" N, 127°41'55" E, 10 September 2007, H.D. Shin, KUS-F22852; Korea, Pocheon, Kookmangbong recreational forest, 38°1'2" N, 127°23'42" E, 29 July 2008, M.J. Park, KUS-F23524 (culture: KACC43869); Korea, Pyeongchang, Jinbu-myon, 37°39'40" N, 128°32'51" E, 21 September 2008, H.D. Shin & M.J. Park, KUS-F23682 (culture: KACC43966); Korea, Hongcheon, Sangoan-ri, 37°37'12" N, 127°47'5" E, 27 October 2008, H.D. Shin & M.J. Park, KUS-F23909 (culture: KACC44083).

Results and discussion

Three *Pyricularia* species have been previously reported as causal agents responsible for blast disease on four *Commelina* species: *P. ebbelsii* M.B. Ellis on *C. africana* (Ellis 1976), *P. oryzae* var. *commelinae* Thirum. et al. on *C. benghalensis* (Thirumalachar et al. 1956, Hashioka 1973), and *P. grisea* on *C. agraria* (Purchio & Muchovej 1993) and *C. erecta* (Halmos 1970).

The conidia of *P. ebbelsii* are characteristically curved and easily differentiated from those of *P. commelinicola,* which are straight. Sizes of conidia and conidiophores allow the separation of *P. commelinicola* from *P. grisea*. As described by Ellis (1971), *P. grisea* has smaller conidia (17–28 × 6–9 µm) and shorter conidiophores (up to 150 µm long) than those of *P. commelinicola* (27–42(–45) × 10–12.5 µm and 150–530 µm, respectively). *Pyricularia oryzae* var. *commelinae* is clearly distinguished from *P. commelinicola* by its smaller lesions (4–6 mm diam.), fewer septa in the conidiophores (1–2-septate), and shorter conidia (21–30 × 10–13 µm) (Thirumalachar et al. 1956). Although the conidial widths of these two species somewhat overlap, the obclavate conidia and average l/w ratio of 3.1 in *P. commelinicola* are clearly different to the relatively broadly pyriform conidia and average l/w ratio of 2.3 in *P. oryzae* var. *commelinae* (calculated from data of Thirumalachar et al. 1956).

In the present study, phylogenetic analysis of ITS rDNA sequences showed that *Pyricularia* species are separated to several distinct lineages, associated with different hosts, as shown in previous studies (Bussaban et al. 2005, Hirata et al. 2007). In the neighbor-joining tree (Fig. 3) four isolates of *Pyricularia* originating from *C. communis* formed an independent clade with bootstrap value of 100%, indicating a high possibility of them being a phylogenetic species. Unfortunately, *P. ebbelsii* and *P. oryzae* var. *commelinae*, distinguished from the present fungus morphologically, could not be molecularly compared with *P. commelinicola* because living cultures were not available. Molecular

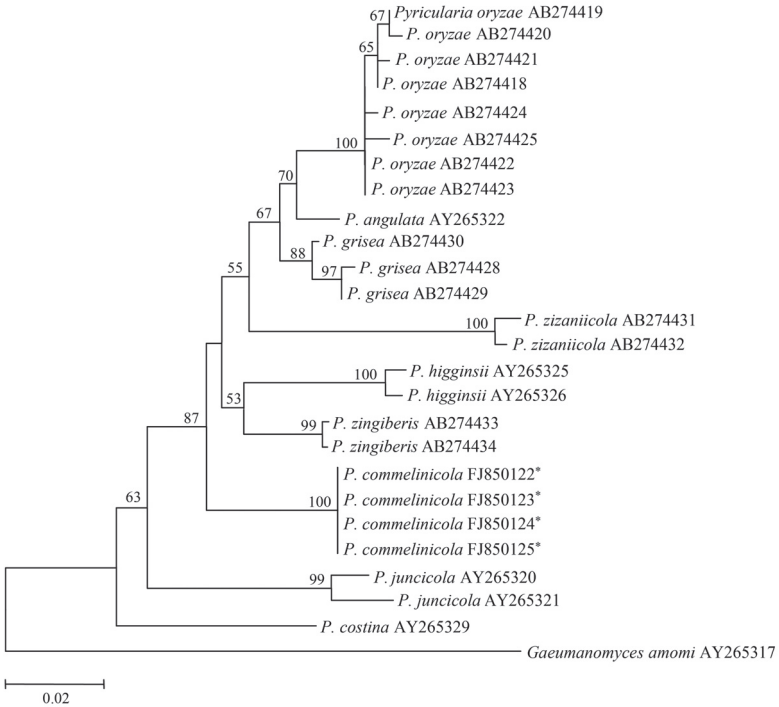

Fig. 3. A neighbor-joining tree of *Pyricularia* spp. based on the ITS rDNA sequences. Bootstrapping values greater than 50% are indicated above the braches (1000 replicates). The number of nucleotide changes between taxa is represented by branch length and a scale bar equals the number of nucleotide substitution per site. Taxa marked with asterisks(*) are sequenced in this study.

data for these two taxa are needed to infer the phylogenetic relationship with *P. commelinicola*. *Pyricularia commelinicola* is, therefore, regarded as a new species based on morphological and ITS-based molecular comparisons.

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